

AMENDMENTS TO THE SPECIFICATION

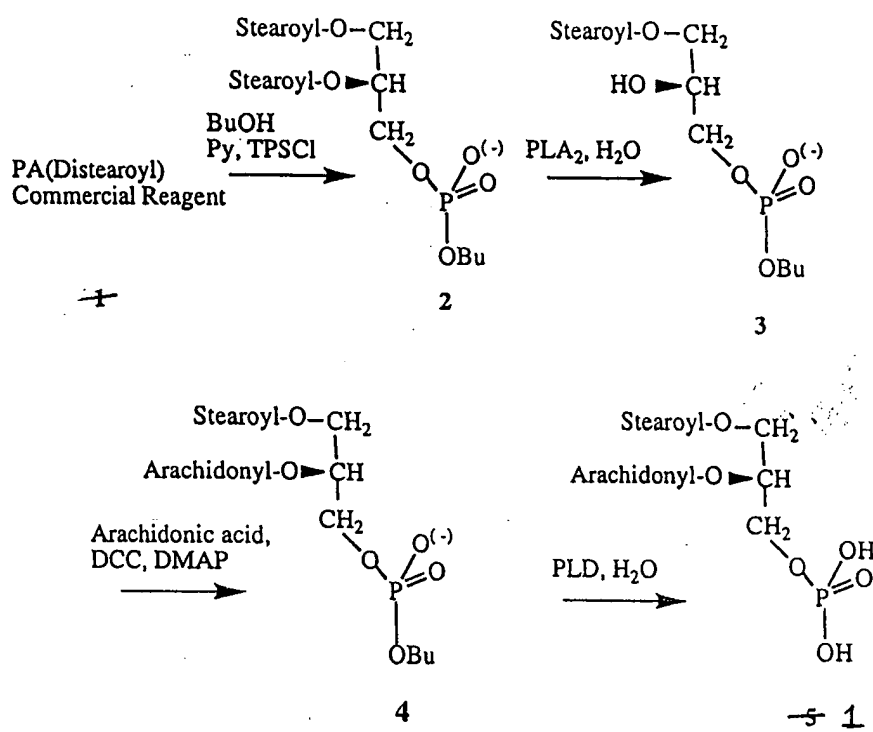
In the specification, at page 8, lines 5-16, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Prior art methods of synthesis of PtdIns have disadvantages inherent in their choice of strategies, reagents and protocols for formation of the phosphodiester bond of PtdIns. In general, literature syntheses create a phosphotriester as precursor intermediate for the phosphodiester bond in one of two ways. Some syntheses employ pentavalent phosphorus reagents which carry a protecting group, usually a phenyl group, that must be removed by catalytic hydrogenolysis to generate the requisite phosphodiester (Young et al., 1990). Hydrogenolysis of phenyl-phosphates causes concomitant reduction of (poly)unsaturated fattyacyl, and of unsaturated bonds in sphingo and related moieties. Most prior art syntheses employ trivalent phosphorus reagents and initially create a phosphite or equivalent intermediate which is then oxidized to the ~~phosphotriester~~ phosphotriester and the latter deprotected to the required phosphodiester. In these syntheses, the reagents and protocols for oxidation damage unsaturated and related groups, particularly (poly)unsaturated fattyacyls.

In the specification, at page 12, lines 1-24, please delete the existing paragraph and scheme and replace with the following paragraph and scheme after implementing the following changes:

Lipid Synthons: Lipid-phosphoric acids are the preferred lipid synthons. The chiral lipid-phosphoric acid synthons represented by the phosphatidic acid 1-stearoyl-2-arachidonyl-*sn*-glycero-3-phosphoric acid **1** (Scheme 2) and its enantiomer 3-stearoyl-2-arachidonyl-*sn*-glycero-1-phosphoric acid **2** (structure not shown) were prepared from the corresponding *n*-butyl esters

by lipolysis with phospholipase D (PLD) as outlined in Scheme 2. Other methods for synthesis of lipid-phosphoric acids are available in the literature and may be utilized. The cited background literature is incorporated herein by reference.



Scheme 2: Synthesis of 1-O-stearoyl-2-O-arachidonyl-*sn*-glycero-3-phosphoric acid (5). (1).

In the specification, from page 13, line 28 to page 14, line 8, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Condensation of Lipid and *myo*-Inositol Synthons: The condensation reaction between the lipid-phosphoric acid and the selectively *O*-protected *myo*-inositol is carried out in ~~an aromatic~~ an aromatic or aliphatic *tert.* amine, using an arylsulfonyl chloride as the phosphoric acid activating reagent. Other activating chemistries and activating reagents, including carbodiimides such as dicyclohexylcarbodiimide, trichloroacetonitrile, and arylsulphonyl-triazoles, may be employed,

or the phosphoric acid may be employed as its phosphoryl chloride or bromide derivative.

Phosphorylation based on the phosphodiester condensation reaction between a lipid-phosphoric acid and an alcohol using pyridine as the *tert.* amine and 2,4,6-triisopropylbenzene-sulfonyl chloride (TPSCI) as the phosphoric acid activating reagent (Aneja et al., 1970) is preferred and this and related literature on phosphodiester synthesis is incorporated herein by reference.

In the specification, at page 14, lines 10-21, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The general protocol for phosphatidylation comprising reaction in anhydrous pyridine solution at r.t. between a phosphatidic acid activated by triisopropylbenzenesulfonyl chloride (TPSCI) was originally developed for primary alcohols (Aneja et al., 1970). Application of this protocol to 1D-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol gave less than 50% yield. Therefore, a new protocol was optimized for chiral secondary alcohols in the *myo*-inositol series. The molar ratios of the reactants, reaction temperature, and the order and rate of addition of phosphatidic acid to the other reactants were found to be critical parameters. Optimization of these ~~parameter~~ parameters produced a dramatic, highly reproducible increase in yield. A typical optimized protocol is given in the section on synthesis from 1D-2,3,4,5,6-pentabenzyl *myo*-inositol. This novel protocol for condensation between lipid-phosphoric acids and the 1-*O*-(equatorial) hydroxyl of *myo*-inositol synthons, gives very high yields (upto 90%) of the (*O*-protected)-inositolphospholipid, and is an integral inventive step in the new approach to synthesis of inositolphospholipid.

In the specification, at page 17, lines 22-25, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Reaction between 1L-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol **8** (not shown) and dioctanoyl-*sn*-3-PA **9** ($R^1CO = R^2CO = n\text{-octanoyl}$) and deprotection gave **14**, the 1L-*myo*- diastereomer of **5 12** ($R^1CO = R^2CO = n\text{-octanoyl}$) (structure not shown) and DL-2- PtdIns identical with **13** ($R^1CO = R^2CO = n\text{-octanoyl}$).

In the specification, at page 17, lines 26-30, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Compound 14 (structure not shown): *1L-1-(1, 2-Dioctanoyl-sn-glycero-3-phospho)-myo- inositol*: $[\alpha]_D +12.85$ (c 0.50, CHCl_3 - CH_3OH 4:1); m/z 585.0 ($M-H$); ^1H -, ^{13}C -NMR and ^{31}P -NMR virtually identical with 1D-*myo*- diastereomer **5 12** ($R^1CO = R^2CO = n\text{-octanoyl}$). ^{31}P -NMR varied somewhat with conditions. Notably, mixtures of 1D-*myo*- **12** and 1L-*myo*- diastereomer **14** always showed a single sharp ^{31}P -NMR peak.

In the specification, from page 19, line 25 to page 20, line 7, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Synthesis of Inositolphospholipids with Unsaturated Lipid Residues: Synthesis of various phosphatidyl-*myo*-inositols with unsaturated fattyaclys, ceramide-phospho-*myo*-inositols with unsaturation in the sphingo-lipid residue was illustrated (*vide supra*) using 2,3:5,6-*O*-protected-*myo*-inositol as the inositol synthon. Analogous ~~synthesis~~ syntheses are achieved using 3,4,5,6-tetra-*O*-protected-*myo*-inositol and 2,3,4,5,6-penta-*O*-protected-*myo*-inositol

synthons wherein the protecting group is chosen from the group comprising but not limited to 4-methoxybenzyl, 9-fluorenylmethyl, tetrahydropyranyl, tetrahydrofuranyl, chloroacetyl, levulinoyl, and related moieties which do not require Pd metal catalyzed hydrogenolysis for deprotection. Thus, reaction between 1D-3,4,5,6-tetra-*O*-(4-methoxybenzyl)-*myo*-inositol and dioleoyl-*sn*-3-PA, and deprotection using moist DDQ yielded 1D-1-(1, 2-dioleoyl-dioleoyl-*sn*-glycero-3-phospho)-*myo*-inositol as the inositolphospholipid. Similarly, reaction using 1D-2,3,4,5,6-penta-*O*-tetrahydropyranyl-*myo*-inositol, and deprotection using acetic acid-water (80:20) at 65 °C gave 1D-1-(1, 2-dioleoyl-*sn*-glycero-3-phospho)-*myo*-inositol.

In the specification, from page 20, line 9 to page 21, line 23, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Novel Inositolphospholipid molecular Species and Applications: The present methods have provided for the first time, novel pure individual molecular species of phosphatidyl-*myo*-inositols with unequivocal stereo-structures, carrying combinations of one saturated and one polyunsaturated fattyacyl, or a single polyunsaturated fattyacyl at both glycerol hydroxyls. The need for and non-availability of such PtdIns molecular species was highlighted earlier under “Related Art” and “Summary of the Invention.” Preferred polyunsaturated fattyacyls include the diunsaturated octadecadienoate isomers linoleoyl, and conjugated-linoleoyl, octadecatrienoate isomers alpha- and ~~gamma-linoleoyl~~, gamma-linolenoyl, eicosanoyl, eicosenoyl, eicosadienoyl, ~~eicosatrienoyl~~, eicosatrienoyl, arachidonyl, eicosapentaenoyl, and docosahexaenoyl, because the corresponding free fatty acids have favorable pharmacodynamic properties, including anticancer and antiinflammatory effects. Parinaric acid is fluorescent and provides a PtdIns which is well suited for highly sensitive analysis by fluorescence techniques.

Preferred PtdIns include 1D-1-(1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phospho)-*myo*-inositol, 1D-1-(1-stearoyl-2-conjugated linoleoyl-*sn*-glycero-3-phospho)-*myo*-inositol, 1D-1-(1-stearoyl-2-eicosapentatenoyl-*sn*-glycero-3-phospho)-*myo*-inositol, and 1D-1-(1,2-di(conjugated linoleoyl)-*sn*-glycero-3-phospho)-*myo*-inositol. These novel PtdIns have utility as biochemical precursors of polyunsaturated fatty acids of the eicosanoid cycle, and diacylglycerol second messengers, formed respectively by the action of phospholipase A₂ and PtdIns-specific phospholipase C enzyme families. This is simulated in Example 1 by the action of phospholipase A₂ on 1D-1-(1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phospho)-*myo*-inositol which liberated arachidonic acid. 1D-1-(1-Stearoyl-2-arachidonoyl-*sn*-glycero-3-phospho)-*myo*-inositol is the major species among numerous molecular species with different fattyacyls which occur in animal tissues derived PtdIns. For convenience, these are often referred to in biochemical literature as stearoyl-arachidonoyl-PtdIns, and sometimes erroneously labeled as such in biochemical reagent catalogues. However, the pure molecular species 1D-1-(1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phospho)-*myo*-inositol has not been prepared previously. Novel analogues wherein the stearoyl residue in 1D-1-(1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phospho)-*myo*-inositol is replaced by an -amino-alkyl, -amino-alkanoyl or other -reactive group-alkyl residue and the arachidonoyl is retained or replaced by other mono- or polyunsaturated fattyacyl, are useful intermediates for attaching reporter groups and for tethering to polymer and metal supports. Further, analogues in which a part or all of one or both alkyl chains are replaced by polyethylene glycol (PEG) residues are useful as water soluble congeners of ~~inositol~~ inositolphospholipids. The aforementioned analogues are obtainable only by the present approach to synthesis. PtdIns encounter aqueous media in most applications and nature of the hydrates determine suitability, particularly ease of use in any application. These novel PtdIns hydrate readily to liquid crystalline phases, as

exemplified in Example 9 for 1D-1-(1-stearoyl-2-arachidonyl-*sn*-glycero-3-phospho)-*myo*-inositol, and thus have advantages over long straight chain saturated PtdIns which are comparatively difficult to hydrate and often form intractable hydrates. The structural types of the liquid crystalline phases are established by X-ray crystallographic analysis of the hydrated lipids. Finally, the high purity PtdIns molecular species are analytical reference standards for analysis of natural PtdIns in tissues and cells by various mass spectral modes, especially ion trap electrospray for quantitation. In this application, the 1-stearoyl-2-eicosdienoyl- and 1-stearoyl-2-eicosatrienoyl type PtdIns are found to be extremely valuable as internal references for quantitation of cellular and ~~tissue~~ tissue as their molecular masses differ by 4 and 2 units from 1-stearoyl-2-arachidonyl-PtdIns which is the single most ~~abundant~~ abundant molecular species in cells.

In the specification, from page 21, line 25 to page 22, line 8, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Synthesis by the new approach, particularly Scheme 5, provided authentic reference samples of pure diastereomers of PtdIns. The structures and absolute stereochemistry of these PtdIns follow directly from the synthetic methods and the known absolute configurations of *sn*-3-phosphatidic acids and *O*-protected-*myo*-inositol synthons, and were supported by ^1H , ^{13}C and ^{31}P NMR spectra. As anticipated, ^1H -NMR of DL-2-PtdIns show tD signal at δ 4.66, 4.68 ($J_{\text{HCCH, cis}}$ 2.44 & 2.1; J_{HCOP} 8.24) which is characteristic of the 2-H in a 2-phospho-*myo*-inositol, the 1-H & 3-H, and 4-H & 6-H resonances show overlapping two proton signals, and can be distinguished from the 1D-1- (or 1L-1-) PtdIns. ^{31}P -NMR of DL-2- and 1D-1- mixtures show single peaks for each ingredient. Further, the structurally isomeric 1- and 2-PtdIns were separated by TLC on

boric acid impregnated silica plates. Thus contamination of 1D-1- (or 1L-1-) PtdIns with DL-2-PtdIns can be detected and measured easily by the multifarious criteria established in the present invention. None was found in synthetic 1D-1- PtdIns prepared according to the present invention.

In the specification, at page 22, lines 10-14, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Authentic reference samples of 1D-1- and 1L-1- PtdIns prepared by methods of the present invention could not be separated by chromatography, and their ^1H , ^{13}C , and ^{31}P NMR spectra showed no significant differences. Critically, the ^{31}P NMR spectrum of a mixture of 1D-1- and 1L-1- PtdIns showed a single peak. Thus, optical rotation emerges as the cardinal stereochemically significant parameter for diastereomer ~~characterization~~. characterization.

In the specification, from page 22, line 16 to page 23, line 2, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The various PtdIns differ in fattyacyl type and hence molecular weight. To normalize for these differences in the homologous series, we have proposed comparison of molar rotations $[\phi]$ using the value +51 obtained for natural PtdIns as the bench mark for 1D-1-(*sn*-3-phosphatidyl)-*myo*-inositol absolute configuration (Aneja and Aneja, 2000). $[\phi]$ values for our authentic 1D-1-dioctanoyl PtdIns and 1D-1-distearoyl PtdIns are +51.57 and +51.70 respectively. These values are identical with the bench mark and thus confirm the 1D-1-(*sn*-3-phosphatidyl)-*myo*-inositol absolute configuration. The molar rotation values calculated from the published $[\alpha]_D$ for 1D-1-

PtdIns are significantly larger than +51, and suggest contamination with 1L-1-series PtdIns. Contrary to recent publications which reported negative sign of rotation for 1L-1-series PtdIns (Garigapati and Roberts, 1993), the reference 1L-1-series PtdIns prepared herein all showed a positive sign of optical rotation with $[\phi] +74.13$. Therefore, $[\phi] +74$ is offered as the bench mark for 1L-1-(*sn*-3-phosphatidyl)-*myo*-inositol series. This suggests that the much higher specific and thereby the molar rotation of the prior art preparations of 1D-1- (*sn*-3-phosphatidyl)-PtdIns are due to contamination with 1L-1-(*sn*-3-phosphatidyl)- PtdIns diastereomers. In contrast, the PtdIns products of the synthesis, characterized by molar rotation equal to the bench-mark values are pure individual diastereomers, and represent uniquely pure preparations of PtdIns.

configuration,

In the specification, at page 23, lines 15-25, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

1D-2,3:5,6-*O*-dicyclohexylidene-*myo*-inositol (systematic name 1L-1,2:4,5-*O*-dicyclohexylidene-*myo*-inositol) and 1L-2,3:5,6-*O*-dicyclohexylidene-*myo*-inositol (systematic name ~~1L-1,2:4,5~~ 1D-1,2:4,5-*O*-dicyclohexylidene-*myo*-inositol) are prepared from *myo*-inositols as described (Aneja et al., 1994). 1D-1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol (common name 1L-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol) and 1L-1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol (common name 1D-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol) are prepared from 1D- and 1L-3-*O*-allyl-1,2:4,5-di-*O*-cyclohexylidene-*myo*-inositols as described (Aneja and Parra, 1994). 1D-1,4,5,6-tetra-*O*-benzyl-*myo*-inositol (common name 1L-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol) and 1L-1,4,5,6-tetra-*O*-benzyl-*myo*-inositol (common name 1D-3,4,5,6-tetra-*O*-benzyl-*myo*-

inositol) are prepared as described (Aneja et al., 1994). All were obtained in >99% enantiomeric ~~enatimeric~~ purity. Lipid synthons were prepared as described in earlier sections.

In the specification, at page 24, lines 1-15, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

1D-1-(1-Stearoyl-2-arachidonyl-sn-glycero-3-phospho)-myo-inositol. 1D-2,3:5,6-*O*-dicyclohexylidene-*myo*-inositol, 1-*O*-stearoyl-2-*O*-arachidonyl-*sn*-glycero-3-phosphoric acid (1.5 equiv.) in anhydrous pyridine at 22 °C, was treated with TPSC1 (3 equiv.). After 2 hr the reaction was allowed to warm to r.t. and treated with water (1.2 equiv.), and evaporated to near dryness in a vacuum, treated with cold water (3 equiv.) and again evaporated to dryness. The crude product so obtained was purified by chromatography on silica eluted with a gradient of CHCl₃ - CH₃OH - NH₄OH, gave the more polar of two as the main product, 1D-1-(1-stearoyl-2-arachidonyl-*sn*-glycero-3-phospho)-~~2,3,5,6-*O*-dicyclohexylidene~~2,3:5,6-*O*-dicyclohexylidene *myo*-inositol, yield 45%. The latter was heated in *t*-BuOH, water and *p*-TSA at 70 °C, and the deprotected product purified by chromatography on silica ¹H NMR showed aromatic peaks for residual *p*-TSA. Deprotection was next effected by heating in ethanol using Nafion, a polymeric sulphonic acid, as catalyst. Chromatography gave pure 1D-1-(1-Stearoyl-2-arachidonyl-*sn*-glycero-3-phospho)-*myo*-inositol (StArach-PtdIns), yield 76%, -m/z 885.6 (M-H)-; ³¹P d 0.422 ppm; [α]_D + 5.48 (c 0.40, CHCl₃ - CH₃OH 4:1); fattyaclys by GC of methyl esters from methanolysis, and PLA₂: 1,2-fattyaclys: 18:0 51.9%, 20:4 49.05%; 2-fattyaclys: 18:0 4.1%, 20:4 95.7% (see below).

In the specification, at page 24, lines 17-29, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Lipolysis of 1D-1-(1-Stearoyl-2-arachidonyl-sn-glycero-3-phospho)-myo-inositol
Catalyzed by Phospholipase A₂: A dispersion of 1D-1-(1-Stearoyl-2-arachidonyl-sn-glycero-3-phospho)-myo-inositol (11 mg) in 0.4 ml of 0.025 M Na₂B₄O₇ buffer containing 0.72 mM CaCl₂, prepared as above, diethyl ether (2 ml) and phospholipase A₂ (from *Crotalus adamanteus*, .2 mg in 0.1 ml buffer) was stirred at 30-31 °C for 6 hr. The mixture was acidified (pH 3) with 0.02 M ~~HCl~~ HCl, the lipids extracted into CHCl₃, and treated with diazomethane solution to convert free fatty acids into methyl esters. The methyl ester fraction was isolated by chromatography over silica in a micropipette eluted with hexane-ether (9:1) and subjected to GC analysis using an HP-225 0.25 mm diam X 30 m film capillary column at 180-220 °C. Composition of the fatty acid methyl esters: stearate 4.1%, arachidonate 95.7% represents the 2-fattyacyl composition. The total fattyacyl determined by GC of methyl esters from acid-catalyzed methanolysis, representing both the 1- and 2-fattyacyls was: stearate 51.9%, arachidonate 49.05%.

In the specification, at page 25, lines 3-11, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

1D-1-(1,2-Dioctanoyl-sn-glycero-3-phospho)-2,3,4,5,6-penta-O-benzyl-myo-inositol. A solution of 1,2-dioctanoyl-sn-glycero-3-phosphoric acid and 1D-2,3,4,5,6-penta-O-benzyl-myo-inositol (systematic name 1L-1,2,4,5,6-penta-O-benzyl-myo-inositol) in anhydrous pyridine at r.t. was treated with triisopropylbenzene sulphonyl chloride (TPSCl) (molar ratios 2:1:4). After 3 hr., water (excess) was added, the mixture evaporated to dryness in a vacuum and the residue

extracted with ether. The ether soluble fraction was purified by chromatography on silicagel eluted with a gradient of CHCl₃ - CH₃OH - NH₄OH, gave 1D-1-(1,2-dioctanoyl-*sn*-glycero-3-phospho-2,3,4,5,6-penta-*O*-benzyl)-~~myo~~-inositol, phospho)-2,3,4,5,6-penta-*O*-benzyl-~~myo~~-inositol, yield 78%, +m/z 1037.4 (M+H), $[\alpha]_D + 13.13$ (c 0.8, CHCl₃).

In the specification, at page 25, lines 15-19, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

*1L-1-(1,2-Dioctanoyl-*sn*-glycero-3-phospho)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol.*

This was prepared from 1L-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (systematic name 1D-1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol) exactly as described above for 1D-1-(1,2-dioctanoyl-*sn*-glycero-3-phospho)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol. Yield 81%, +m/z 1037.4 (M+H), $[\alpha]_D - 7.023$ (c 0.8, CHCl₃).

In the specification, from page 25, line 23 to page 26, line7, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

*1D-1-(1,2-Dioctanoyl-*sn*-glycero-3-phospho)-*myo*-inositol.* 1D-1-(1,2-dioctanoyl-*sn*-glycero-3-phospho)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol and Pd-black (catalyst) in *tert*-butanol at room temperature was shaken in H₂ gas at 50 psi for 36 hr. The catalyst was removed by filtration, and the filtrate evaporated to dryness in a vacuum. The residue was pure 1D-1-(1,2-dioctanoyl-*sn*-glycero-3-phospho)-*myo*-inositol, yield 100%; $[\alpha]_D + 8.90$ (c 0.45, CHCl₃ - CH₃OH 4:1); ES(-) MS m/z 585.0 (M-H)-; ¹H-NMR (400 MHz, CDCl₃-CD₃OD, 2:1) d ppm 0.90 (t, 6H, CH₃), 1.29 (br s, 16H, (CH₂)₈), 1.61 (*m*, 4H, CH₂CH₂C=O), 2.29-2.36 (*m*, 4H, CH₂C=O), 3.30 (*t*, 1H, inositol 5-H), 3.46-3.48 (dd, 1H, inositol 3-H), 3.67(*m*, 1H, inositol

4-H), 3.80 (m, 1H, inositol 6-H), 3.92 (t, 1H, inositol 2-H), 4.05-4.17 (m, 2H, *sn*-3 CH₂), 4.45 (m, 2H, *sn*-1 CH₂), 4.28 (t, 1H, inositol 1-H), 5.26 (m, 1H, *sn*-2 CH); ¹³C-NMR (100 MHz, CDCl₃-CD₃OD, 2:1) 173.69 & 173.35 (2 C=O), 76.24 (inositol C-1), 74.12 (inositol C-5), 72.10 (inositol C-4), 71.35 (inositol C-6), 71.19 & 70.98 (inositol C-2), 70.14 & 70.06 (*sn*-2 glycerol C), 63.46 (*sn*-3 glycerol C), 62.39 (*sn*-1 glycerol C), 33.82 (acyl chain *sn*-2 α-CH₂), 33.68 (acyl chain *sn*-1 α-CH₂), 31.29, 31.28, 24.51 & 24.45 ((CH₂)_n), 22.19 (acyl chain ω-CH₂), 13.46 (acyl chain CH₃); ³¹P-NMR (162 MHz, CDCl₃-CD₃OD, 2:1) δ ppm (ext. H₃PO₄) -0.259 (s).

In the specification, at page 26, lines 11-17, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

1L-1-(1,2-Dioctanoyl-sn-glycero-3-phospho)-myo-inositol. Hydrogenolysis of 1L-1-(1,2-dioctanoyl-*sn*-glycero-3-phospho)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol as described above for the *1D-1-* analogue gave 1L-1-(1,2-dioctanoyl-*sn*-glycero-3-phospho)-*myo*-inositol, yield 100%, -m/z 585.0 (M-H), [α]_D + 12.3 (c .9, CHCl₃ - CH₃OH 9:1); ¹H-, ¹³C-NMR and ³¹P-NMR virtually identical with 1D-*myo*- diastereomer (~~Example 8~~ Example 4); δ ³¹P-NMR varied somewhat with conditions. Notably, mixtures of 1D-*myo*- and 1L-*myo*- diastereomer always showed a single sharp ³¹P-NMR peak.

In the specification, at page 26, lines 21-30, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

DL-2-(1,2-Dioctanoyl-sn-glycero-3-phospho)-3,4,5,6-tetra-O-benzyl-myoinositol. A solution of 1,2-dioctanoyl-*sn*-glycero-3-phosphoric acid and 1D-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (systematic name 1L-1,4,5,6-tetra-*O*-benzyl-*myo*-inositol) in anhydrous pyridine at r.t.

was treated with triisopropylbenzene sulphonyl chloride (TPSCl) (molar ratios 1:1:2). After 3 hr., water (excess) was added, the mixture evaporated to dryness in a vacuum and the residue extracted with ether. The ether soluble fraction was purified by chromatography on silicagel eluted with a gradient of CHCl_3 - CH_3OH - NH_4OH , gave 1D-1-(1,2-dioctanoyl-*sn*-glycero-3-phospho)-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol, yield 48%, $-m/z$ 945.2 (M-H), $[\alpha]_D - 2.15$ (c 1.4, CHCl_3), followed by DL-2-(1,2-dioctanoyl-*sn*-glycero-3-phospho)-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol, yield 38%, $-m/z$ 945.2 (M-H), $[\alpha]_D + 18.64$ (c 0.8, CHCl_3).

In the specification, at page 27, lines 3-12, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

DL-2-(1,2-Dioctanoyl-*sn*-glycero-3-phospho)-~~7 α -inositol~~ *myo*-inositol. DL-2-(1,2-dioctanoyl-*sn*-glycero-3-phospho)-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol and Pd-black (catalyst) in *tert*-butanol at room temperature was shaken in H_2 gas at 50 psi for 36 hr. The catalyst was removed by filtration, and the filtrate evaporated to dryness in a vacuum. The residue was pure DL-2-(1,2-dioctanoyl-*sn*-glycero-3-phospho)-*myo*-inositol, yield 100%; $[\alpha]_D + 7.25$ (c 0.50, CHCl_3 - CH_3OH 4:1), $-m/z$ 585.0 (M-H)-; ^1H -NMR (400 MHz, CDCl_3 - CD_3OD , 2:1) δ ppm 0.89 (t, 6H, CH_3), 1.29 (br s, 16H, $(\text{CH}_2)_8$), 1.61 (m, 4H, $\text{CH}_2\text{CH}_2\text{C}=\text{O}$), 2.33 (m, 4H, $\text{CH}_2\text{C}=\text{O}$), 3.28 (t, 1H, inositol 5-H), 3.52 (dT, 2H, inositol 1-H & 3-H), 3.63(m, 2H, inositol 4-H & 6-H), 4.17 (m, 1H, *sn*-1 CH_2), 4.22 (m, 2H, *sn*-3 CH_2), 4.40 (m, 1H, *sn*-1 CH_2), 4.68 (tD, 1H, inositol 2-H), 5.26 (m, 1H,*sn*-2 CH); ^{31}P -NMR (162 MHz, CDCl_3 - CD_3OD , 2:1) δ ppm (external H_3PO_4 ref.) - 0.701 (s).

In the specification, at page 27, lines 16-19, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

1D-1-(1,2-Distearoyl-sn-glycero-3-phospho)-myo-inositol. Hydrogenolysis of 1D-1-(1,2-distearoyl-*sn*-glycero-3-phospho)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol as described above for the dioctanoyl series gave 1D-1-(1,2-distearoyl-*sn*-glycero-3-phospho)-*myo*-inositol, yield 100%, -*m/z* 865.6 (M-H), $[\alpha]_D + 5.9$ (c 0.2, CHCl₃ - CH₃OH 9:1).

In the specification, from page 27, line 23 to page 28, line 5, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Hydration Behavior of 1,2-Disaturated-fattyacyl and 1-Saturated-fattyacyl-2-Polyunsaturated-fattyacyl type PtdIns. The mesogenic behavior of 1D-1-(1-stearoyl-2-arachidonyl-*sn*-glycero-3-phospho)-*myo*-inositol versus 1D-1-(1,2-distearoyl-*sn*-glycero-3-phospho)-*myo*-inositol was compared. A solution of each lipid (5 mg) in CHCl₃ was evaporated to obtain a thin film in a small test tube. The residue was left under a high vacuum for 12 hr to remove traces of solvent. Pure water (0.2 ml, HPLC grade) was added, the tube contents mixed by agitation over a vortex mixer and in bath type sonicator under Argon gas at 35-36°C. The resulting dispersion was examined on a microscope slide under crossed polarizers in a microscope (Small, 1986). Sample prepared with 1D-1-(1-stearoyl-2-arachidonyl-*sn*-glycero-3-phospho)-*myo*-inositol appeared as a clear fluid birefringent phase similar to the myelin figures formed by egg yolk phosphatidylcholine. Sample prepared with 1D-1-(1,2-distearoyl-*sn*-glycero-3-phospho)-*myo*-inositol showed undispersed powder-like material.

In the specification, at page 29, lines 13-14, please delete the existing two lines and replace with the following two lines after implementing the following changes:

Aneja, R.; Aneja, S. G.; ~~Para~~ Parra, A. **1995**, *Tetrahedron Asymmetry*, **6**, 17-18.

Aneja, R.; Aneja, S. G.; ~~Para~~ Parra, A. **1996**, *Tetrahedron Lett.* **37**, 5081-5082.